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13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

The vaccination efficacy of DNA encoding autologous rat ErbB-2 (neu) or heterologous human ErbB-2 (Her-2) is compared in rat neu transgenic mice (BALB neuT). Cross reactivity between Her-2 and rat neu was tested initially by immunizing normal mice, twice, i.m. with pEFBosGM-CSF and pCMVE2TM or pCDneuTM. E2TM and neuTM encodes the extracellular (ECD) and transmembrane (TM) domains of Her-2 and neu, respectively. Immunized mice were challenged with mammary tumor D2F2 expressing Her-2 (D2F2/E2) or neu (D2F2/neu). All mice immunized with E2TM or neuTM rejected tumors expressing the corresponding antigen. There is significant cross-protection against tumors expressing the non-corresponding ErbB-2, although the antibodies demonstrated little cross-reactivity. When tested in neuT mice which are tolerant to rat neu, neuTM but not E2TM delayed spontaneous tumorigenesis. Therefore, Her-2 and rat neu are cross-reactive antigens in normal mice, but only autologous neu, not heterologous Her-2 induced protective immunity in NeuT transgenic mice. This may indicate a lack of cross-reactivity of antibodies which are critical in inhibiting NeuT tumor. To enhance the immunogenicity of autologous ErbB-2, an adjuvant sequence of Pan DR Reactive Epitope (PADRE) has been cloned into human ErbB-2 and the immunogenicity is being tested.

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INTRODUCTION

The goal is to develop vaccination regimen to a tumor associated self-antigen, ErbB-2 and induce significant anti-tumor immunity in BALB NeuT mice which are tolerant to rat ErbB-2 (neu). The objectives are to

(1) Measure vaccination efficacy after depletion of negative regulatory $CD25^{+}CD4^{+}$ cells.
(2) Compare immune reactivity and tumor growth inhibition in ErbB-2 transgenic mice vaccinated with autologous or heterologous ErbB-2
(3) Construct and test vaccination efficacy of ErbB-2 containing Pan DR Reactive Epitope (PADRE).

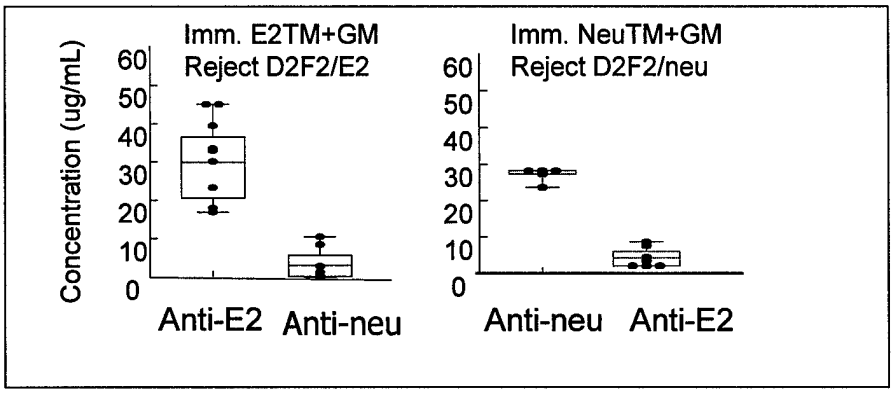
BODY

Immunization with DNA encoding tumor-associated antigen has demonstrated striking efficacy in mice [1,2]. Many tumor-associated antigens are, however, normal molecules over-expressed or inappropriately expressed. There may exist self-tolerance to such tumor associated antigens. To test the hypothesis that immunization with DNA vaccines encoding heterologous antigens can overcome tolerance and achieve anti-tumor effect, the cross-reactivity of human (Her-2) and rat ErbB-2 (neu) was first defined in BALB/c mice (Table 1). Mice were immunized twice by i.m. injection with 100 μg each of pCMVE2TM and pEFBos GM-CSF. At two wks following the last immunization, mice were challenged by s.c. injection with 2×10^5 D2F2 cells expressing human ErBB-2 (D2F2/E2) or rat neu (D2F2/neu). There was significant protection in all immunized mice, although complete protection was achieved only with autologous ErbB-2 vaccine. In mice immunized once and challenged with a TUBO cell line which was derived from a spontaneous tumor in NeuT transgenic female mice, only neuTM immunized mice were protected.

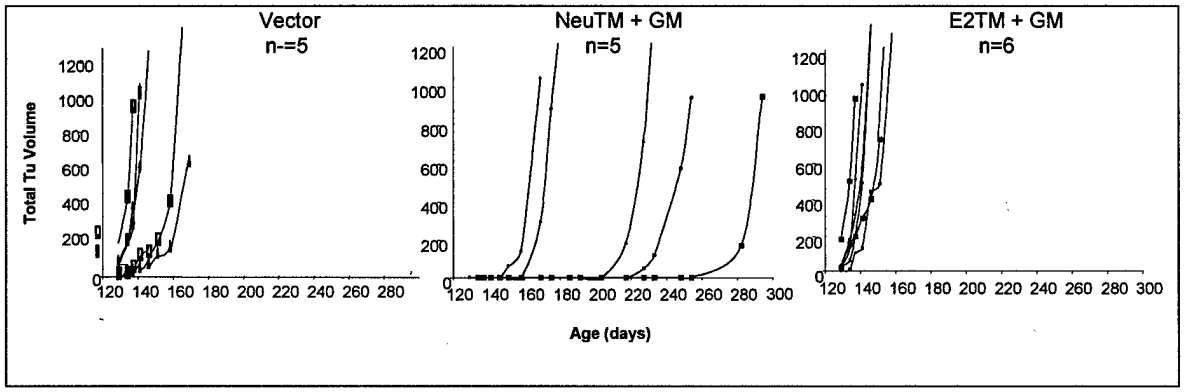
Table 1 Cross reactivity of Human and rat ErbB-2 in BALB/c mice

DNA vaccine, 2X	D2F2 tumor Expressing	Tu incidence	DNA vac, 1X. Challenge with	Tu incidence
Blank vector	Human ErbB-2	8/8		
pE2TM + pGM	Human ErbB-2	0/7		
pneuTM +p GM	Human ErbB-2	1/6		
Blank vector	Rat Neu	8/8	Tubo	3/3
pE2TM +p GM	Rat Neu	3/7	Tubo	3/3
pneuTM + pGM	Rat neu	0/7	Tubo	0/3

D2F2 cells are highly tumorigenic before ErbB-2 transfection and are independent of ErbB-2 for survival, whereas TUBO cells induced by transforming neu may depend on Neu for proliferation signals. Anti-ErbB-2 antibodies are not critical for D2F2/E2 or D2F2/neu rejection [1], but are primary effectors in TUBO rejection[3]. The inability to reject TUBO cells in mice immunized with pE2TM and pGM-CSF may indicate that anti-Her-2 antibody does not recognize rat neu. To test antibody cross-reactivity, sera was collected from mice which were immunized and had rejected D2F2 tumor expressing the corresponding antigen (Figure 1). Approximately 30 μg/ml of antibody was induced to the respective antigen, with little recognition of the heterologous Her-2 or Neu. This finding is both surprising and interesting and may indicate that ErbB-2 cross reactivity in BALB/c mice is mediated primarily by T cells.



Following this observation, it was expected that the spontaneous tumorigenesis in NeuT female mice would be suppressed by anti-Neu, but not anti-Her-2 antibody. BALB neuT females were immunized with DNA encoding GM-CSF combined with (A) blank vector (B) pE2TM or (C) pNeuTM (Figure 2). Total volume of the spontaneous tumors in each mouse was recorded. Significant delay in tumorigenesis was observed in NeuT mice immunized with pNeuTM, but not pE2TM.



Therefore, to overcome ErbB-2 tolerance, autologous antigen is more effective than heterologous antigen. To enhance the immunogenicity of ErbB-2, Pan DR Reactive Epitope (PADRE) sequence has been inserted into human ErbB-2 DNA and the vaccination efficacy is currently being tested.

KEY RESEARCH ACCOMPLISHMENTS

1. Define cross-reactivity of Her-2 and Neu in BALB/c mice
2. Demonstrate that vaccine induced anti-Her-2 or anti-neu antibody recognize only the autologous antigen.
3. Demonstrate that spontaneous tumorigenesis in BALB NeuT mice can be inhibited by immunization with rat neu, but not Her-2 DNA.

REPORTABLE OUTCOMES

Wei-Zen Wei, John Zielinski, Carmen Kelly, Katia Boggio*, Stefania Rovero* and Guido Forni*, "Autologous, but not heterologous, DNA vaccine inhibits spontaneous mammary tumorigenesis", DOD Era of Hope Conference, Orlando, Fl, 2002

CONCLUSIONS

Her-2 and rat neu are cross-reactive antigens in mice, but only autologous neu, not heterologous Her-2 induced protective immunity in NeuT transgenic mice and may indicate that vaccine induced antibodies recognize only autologous ErbB-2. To enhance the immunogenicity of autologous ErbB-2, an adjuvant sequence of Pan DR Reactive Epitope (PADRE) has been cloned into human ErbB-2 and the immunogenicity is being tested.

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